Effects of temperature on the dose-response curve of HeLa S3 cells irradiated with X-rays

by

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HeLe 細胞の X 線感受性に対する温度効果

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in vitro 培養細胞のコロニー形成能におよぼすX線の作用を，照射中の温度を変えて検討した．その結果線量効果関係の傾斜 D_0 は，照射中の温度10°，23°，26°，37℃ではそれぞれ 153, 103, 108, 90rad であり外挿数はそれぞれ 2.5, 2.0, 2.0, 2.5であつた．つまり HeLa 細胞を 250 kVp, 1.45mmCu, 線量率62 rad/分のX線で照射すると，照射中に低温であるほど生存率は高い．

Introduction

In order to analyse the processes of radiation damage, it is of value to study the effect of temperature on the surviving fraction of biological materials following irradiation. Many workers have reported on the effects of temperature under various conditions[1)-5)], but the mode of response depends on the type of biological material used[6)-8)], and time of treatments[3)9)]. This is still a matter requiring further research.

In this paper, the effect of temperature during X-irradiation on the surviving fraction of HeLa S3 cells is reported.

Materials and Methods

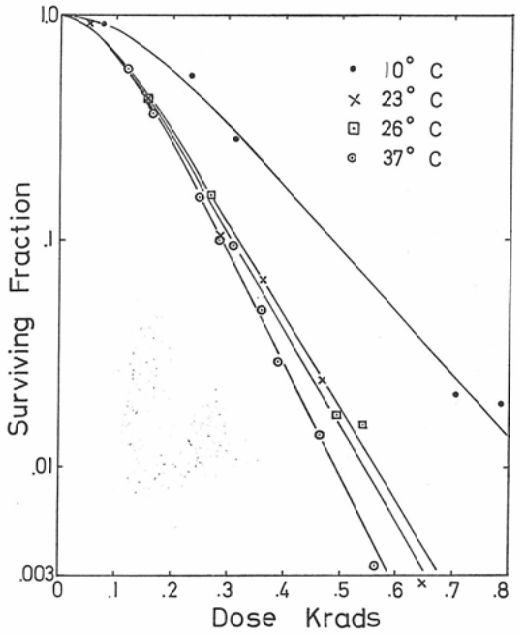
Methods of culture and handling of the cells were essentially the same as that described by Puck and his colleagues[10)11)]. A subclone of HeLa S3 cells and Eagle MEM[12)] supplemented with 20% calf serum were used in all experiments. After treatment with 0.1% trypsin, cell suspension was diluted with growth media to inactivate trypsin digestion.

Concentration of cells was determined with a haemocytometer under a phase-contrast microscope. An adequated number of cells were transplanted into the media in glass dishes to yield about the same number of survivors at each dose level as expected in the controls. After incubation at 37 °C in relatively high humidity for 4 to 5 hours to allow the cells to attach themselves to the bottom of the dish, the cells were irradiated. Platings were kept at 10°, 23°, 26° and 37°C for 5 minutes prior to, during and for 2

minutes after irradiation.

After irradiation in air, culture was continued for a peroid of 14 days at 37 °C in an incubator with a humidified atmosphere of 5% CO_2. The cells were then fixed and stained with crystal violet. Colonies having more than 50 normal cells were counted and regarded as reproductively intact[13]. X-ray exposure was made at 250 kVp with HVL of 1.45 mm Cu and dose rate of 62 rads per minute.

Fig. 1 Dose response curves of HeLa S3 cells irradiated at various temperature



Results and Discussions

Figure 1 shows dose response curves of HeLa S3 cells irradiated with X-rays at 10°, 23°, 26° and 37 °C. All curves displayed a threshold region and a exponential region. The parameter Do of dose response curve at 10°, 23°, 26° and 37 °C estimated from Figure 1 was 153, 103, 108 and 90 rads, and the extrapolation number n was 2.5, 2.0, 2.0 and 2.5, respectively.

A few studies have reported on the effects of temperature on the surviving fraction of mammalian cells, but the effects differ according to the endpoint of radiobiology, biological materials and time of treatment. Weiss reported[5] that the protective effects of low temperature on the surviving fraction of HeLa cells could not be observed and according to Smith and Mckinley, there was a loss of protective effects at low temperature during irradiation.

In the present study, a decreased radiosensitivity of cells was observed at low temperature during irradiation, which is in good agreement with results reported by Belli and Bonte, using X-rays and HeLa cells. The cause of these differences in effects of temperature on radiosensitivity is yet unknown.

It has been noted that at low temperature, 1) cell doubling time is extended[13][14], 2) enzyme activity in cells is depressed, 3) solubility of oxygen increases and 4) speed of diffusion of radicals produced by ionizing radiation will be depressed. The decrease in radiosensitivity of cells at low temperature may be attributable to the difference between the speed of the process from radiochemical reaction to visible

events and that of recovery process from damage. The answer of this possibility must be await future research.

Summary

Using single cell culture method, a decrease in radiosensitibity of HeLa S3 cells at low temperature during X-irradiation was demonstrated. The parameter Do of dose response curves of cells irradiated at 10°, 23°, 26° and 37°C was 153, 103, 108 and 90 rads and the extrapolation number n was 2.5, 2.0, 2.0 and 2.5 respectively.

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